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Development and Validation of a UV Spectrophotometric Method for the Simultaneous Estimation of Lornoxicam and Thiocolchicoside in Combined Dosage Form

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ABSTRACT

A simple, precise, and accurate UV spectrophotometric method was developed and validated for the simultaneous estimation of Lornoxicam and Thiocolchicoside in bulk and pharmaceutical formulations. The method employs methanol as solvent, and absorbance was measured at the respective wavelengths where both drugs showed maximum absorbance with minimal interference. Calibration curves were linear within the concentration ranges of 4–20 µg/mL for Lornoxicam and 5–25 µg/mL for Thiocolchicoside, with correlation coefficients (R^2) of 0.9992 and 0.9995, respectively. Validation was carried out according to ICH Q2(R1) guidelines, including parameters such as linearity, accuracy, precision, LOD, LOQ, robustness, and ruggedness. Recovery studies at 50%, 100%, and 150% levels showed recoveries between 98.7–100.1%, indicating high accuracy. The proposed method is suitable for routine quality control of combined dosage forms of Lornoxicam and Thiocolchicoside.

Keywords: Lornoxicam, Thiocolchicoside, UV Spectrophotometry, Simultaneous Estimation, Method Validation, Pharmaceutical Analysis.

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INTRODUCTION

Lornoxicam is a nonsteroidal anti-inflammatory drug (NSAID) of the oxicam class possessing potent analgesic, anti-inflammatory, and antipyretic properties. It is widely used for the treatment of pain and inflammation associated with osteoarthritis, surgery, and other musculoskeletal disorders (1). Lornoxicam is used for the treatment of various types of pain, especially resulting from inflammatory diseases of the joints, osteoarthritis, surgery, sciatica, and other inflammations. Thiocolchicoside is a semi-synthetic derivative of colchicoside that acts as a muscle relaxant with additional anti-inflammatory and analgesic properties (2-5). Due to their synergistic therapeutic action, both drugs are frequently formulated together in fixed-dose combinations. While chromatographic methods such as HPLC are accurate and sensitive, they are time-consuming and require costly instrumentation (6). In contrast, UV spectrophotometry offers a simpler, economical, and efficient alternative for simultaneous estimation.

Since both drugs are often formulated together, it is necessary to develop a simple and reliable analytical method for their simultaneous estimation. Although chromatographic techniques such as HPLC provide excellent accuracy, they require expensive solvents and instrumentation. UV spectrophotometry, being cost-effective and simple, offers a suitable alternative for simultaneous quantification (9-10). The present study focuses on the development and validation of a UV spectrophotometric method for simultaneous estimation of both drugs in bulk and tablet dosage form according to ICH guidelines (1).

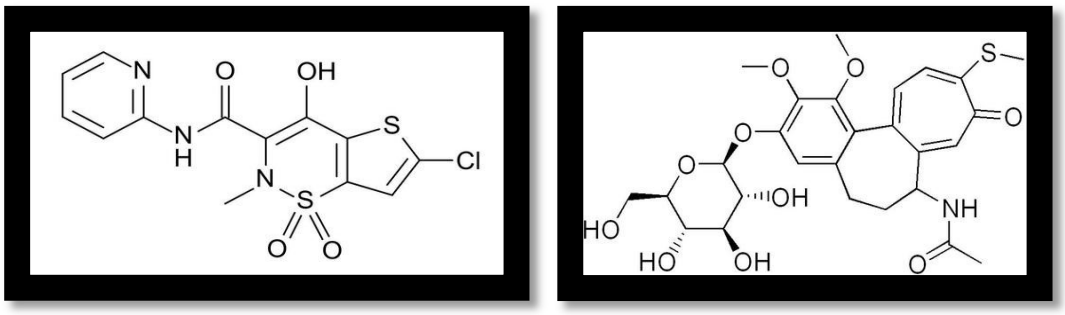


Figure 1: Chemical structures of Lornoxicam and Thiocolchicoside

MATERIALS AND METHOD

Instrument:

All UV measurements were performed using a Shimadzu UV-1800 double-beam spectrophotometer equipped with matched 1 cm quartz cuvettes. Methanol of analytical grade was used throughout the study. Pure Lornoxicam and Thiocolchicoside samples were obtained as gift samples from Seldom Pharma Ltd. All chemicals used were of AR grade and freshly prepared before analysis.

Reagents and Materials:

Pure samples of Lornoxicam and Thiocolchicoside- Methanol (analytical grade) All chemicals and reagents used in the study were of analytical reagent (AR) grade and of 99% purity. Lornoxicam and Thiocolchicoside pure drug samples were obtained as gift samples from Seldom pharma Limited. Methanol (AR grade) was used as the solvent throughout the analysis due to its excellent solubility and compatibility with the selected wavelength range. All solutions were freshly prepared, and proper care was taken to avoid contamination and degradation during the experimental procedures.

Preparation of Standard Stock Solutions:

A stock solution of Lornoxicam and Thiocolchicoside was prepared separately by dissolving 10 mg of each drug in a small volume of methanol, and the volume was made up to 10 mL with the same solvent to obtain a final concentration of 1000 µg/mL. From these stock solutions, working standard solutions were prepared by appropriate dilution with methanol to obtain concentrations ranging from 4–20 µg/mL for both drugs.

For the sample solution, an accurately weighed quantity of the combined tablet formulation equivalent to 10 mg of Lornoxicam and 10 mg of Thiocolchicoside was transferred into a 10 mL volumetric flask, dissolved in methanol, and sonicated for 10 minutes to ensure complete dissolution. The resulting solution was filtered through Whatman filter paper No. 41, and the filtrate was suitably diluted with methanol to obtain the required concentration within the linearity range.

Preparation of sample solution of Lornoxicam and Thiocolchicoside:

For the sample solution, an accurately weighed quantity of the combined tablet formulation equivalent to 10 mg of Lornoxicam and 10 mg of Thiocolchicoside was transferred into a 10 mL volumetric flask, dissolved in methanol, and sonicated for 10 minutes to ensure complete dissolution. The resulting solution was filtered through Whatman filter paper No. 41, and the filtrate was suitably diluted with methanol to obtain the required concentration within the linearity range.

Measurement of Absorbance:

Absorbance values of both standard and sample solutions were recorded at selected wavelengths, and concentrations were calculated using the calibration curves.

Determination of λmax:

The standard stock solutions of Lornoxicam and Thiocolchicoside were appropriately diluted with methanol to obtain concentrations of 10 µg/mL each. These solutions were scanned individually in

the wavelength range of 200–400 nm (4) using a Shimadzu UV-1800 spectrophotometer against methanol as blank. The absorption spectra of both drugs were recorded, and the wavelengths corresponding to maximum absorbance (λmax) were determined.

Lornoxicam exhibited a maximum absorbance at 378nm, while Thiocolchicoside showed its λmax at 368nm. These wavelengths were selected for further analysis as they represent the points of highest sensitivity for each drug. The spectra were examined for any potential overlap, ensuring minimal interference and allowing for accurate simultaneous estimation of both components in the combined formulation.

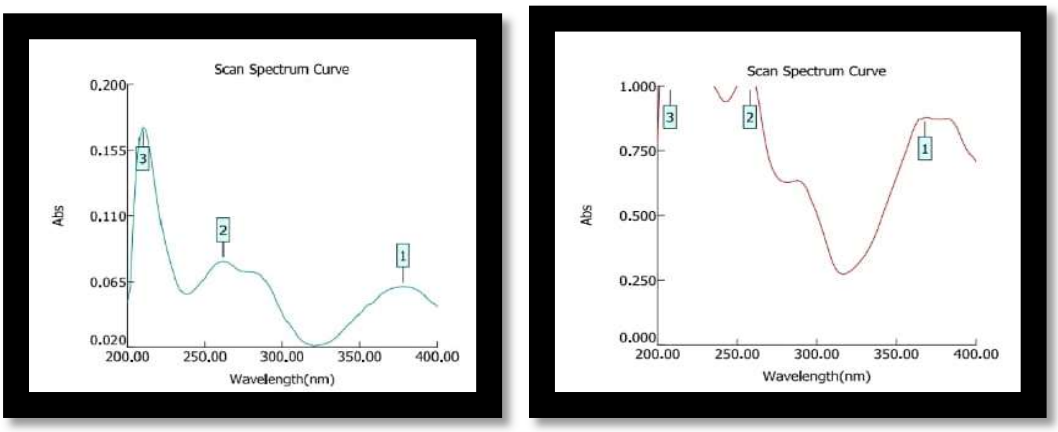


Figure 2: Validation of Method (As per ICH Q2(R1))

Linearity:

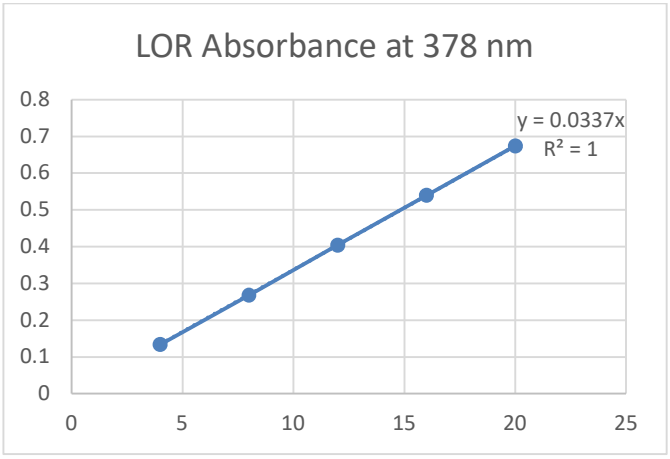
The linearity of the developed UV spectrophotometric method was established by analyzing a series of standard solutions containing Lornoxicam and Thiocolchicoside in the concentration range of 4–20 µg/mL and 5-25 µg/ml (6) . Each solution was scanned at the respective λmax values of 378 nm for Lornoxicam and 368 nm for Thiocolchicoside, using methanol as the blank. The absorbance values obtained were plotted against the corresponding concentrations to construct the calibration curves for both drugs.

The regression analysis of the calibration data demonstrated a strong linear relationship between absorbance and concentration within the selected range, with correlation coefficients (R^2 values) close to unity, confirming the method's linearity. This indicates that the proposed method follows Beer–Lambert's law over the studied concentration range for both analytes, ensuring its suitability for quantitative analysis.

Table 1 & 2: Linearity of Lornoxicam and Thiocolchicoside

Concentration (µg/mL)	LOR Absorbance at 378 nm	Concentration (µg/mL)	THIO Absorbance at 368 nm
4	0.134	5	0.112
8	0.268	10	0.224

12	0.404	15	0.336
16	0.540	20	0.449
20	0.674	25	0.562



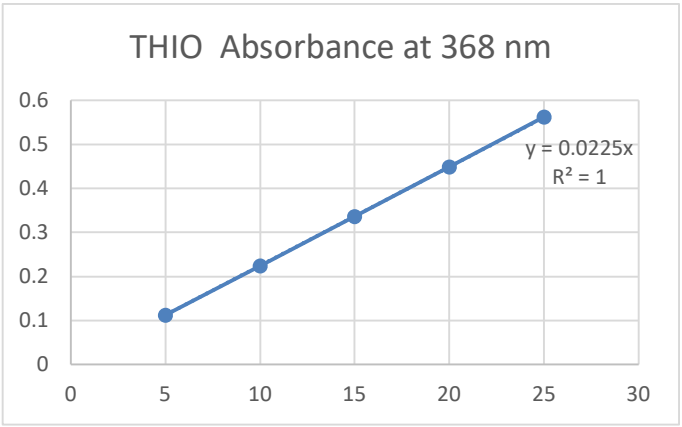


Figure 3: Linearity of Lornoxicam and Thiocolchicoside

Accuracy (Recovery Studies):

The accuracy of the proposed UV spectrophotometric method was assessed by the standard addition method at three concentration levels of 50%, 100%, and 150% of the pre-analyzed sample concentration. Known amounts of pure Lornoxicam and Thiocolchicoside standards were added to a fixed concentration of the sample solution, and the mixtures were analyzed in triplicate at the respective λmax values of 378 nm and 368 nm. The percentage recovery and %RSD were calculated for each level. The obtained results indicated that the recoveries were within the acceptable limits, confirming that the method is accurate, reproducible, and unaffected by formulation excipients.

Table:3 Recovery studies data of LOR

Level of Recovery (%)	Amount Taken (µg/mL)	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	%RSD
50	4	4.0	8.97	99.67	0.58
100	12	12.0	11.97	99.75	0.63
150	20	20.0	14.95	99.67	0.69

Table: 4 Recovery studies data for THIO

Level of Recovery (%)	Amount Taken (µg/mL)	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	%RSD
50	5	5.0	8.96	99.56	0.64
100	15	15.0	11.95	99.58	0.72
150	25	25.0	14.93	99.53	0.75

Precision:

The precision of the proposed method was evaluated by performing intra-day and inter-day studies using standard solutions of Lornoxicam and Thiocolchicoside at three different concentration levels (4,12,20µg/mL) for LOR and (5,15,25µg/mL) for THIO (7). Each concentration was analyzed in triplicate, and the absorbance values were recorded at the respective λmax values of 378 nm and 368 nm.

For intra-day precision, the analysis was carried out three times within the same day at different time intervals, while for inter-day precision, the measurements were repeated on three consecutive days under similar experimental conditions. The results were expressed as % Relative Standard Deviation (%RSD), which was found to be less than 2% for both drugs, indicating that the method is precise and reproducible.

Table 4: Intra-day and Inter-day Precision Data for Lornoxicam

Concentration (µg/mL)	Intra-day Mean Absorbance ± SD	%RSD	Inter-day Mean Absorbance ± SD	%RSD
4	0.268 ± 0.0015	0.56	0.269 ± 0.0021	0.78
12	0.404 ± 0.0023	0.57	0.406 ± 0.0029	0.71
20	0.540 ± 0.0031	0.57	0.541 ± 0.0040	0.74

Table 5: Intra-day and Inter-day Precision Data for Thiocolchicoside

Concentration (µg/mL)	Intra-day Mean Absorbance ± SD	%RSD	Inter-day Mean Absorbance ± SD	%RSD
5	0.224 ± 0.0018	0.80	0.225 ± 0.0022	0.98
15	0.336 ± 0.0024	0.71	0.338 ± 0.0031	0.92
25	0.449 ± 0.0032	0.71	0.450 ± 0.0039	0.87

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined to evaluate the sensitivity of the developed method. These parameters were calculated using the statistical

approach based on the standard deviation (σ) of the response and the slope (S) of the calibration curve, as per ICH Q2(R1) guidelines (8). The following formulae were applied:

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}$$

$$\text{LOQ} = 10 \times \frac{\sigma}{S}$$

The calculated LOD and LOQ values for both drugs are presented in the table below. The low values obtained indicate the high sensitivity of the proposed method, confirming that even small quantities of Lornoxicam and Thiocolchicoside can be accurately detected and quantified using UV spectrophotometry.

Table:6 LOD and LOQ of LOR and THIO

Parameter	Lornoxicam	Thiocolchicoside
Slope (S)	0.0674	0.0562
Standard Deviation (σ)	0.0018	0.0020
LOD (µg/mL)	0.09	0.12
LOQ (µg/mL)	0.27	0.36

Robustness and Ruggedness:

The robustness of the developed method was evaluated by introducing small, deliberate variations in experimental conditions such as wavelength (±2 nm) and solvent composition (±2%), while keeping other parameters constant. Standard solutions of Lornoxicam and Thiocolchicoside were analyzed under these modified conditions, and the resulting absorbance values were compared with those obtained under the optimized conditions. The %RSD values were calculated to assess the impact of these variations. The results showed negligible changes in absorbance and %RSD values less than 2%, confirming that the method remains unaffected by minor experimental fluctuations, thereby demonstrating robustness.

The ruggedness of the method was assessed by performing the analysis of standard solutions by two different analysts on different days using the same instrument and laboratory conditions. The results were expressed as %RSD of the absorbance readings, which were again found to be below 2%, indicating that the method is rugged and reproducible across different analysts and time intervals.

RESULTS AND DISCUSSION:

The present work aimed to develop and validate a simple, accurate, and precise UV spectrophotometric method for the simultaneous estimation of Lornoxicam and Thiocolchicoside in bulk and combined tablet formulations. Methanol was selected as the solvent since both drugs showed good solubility and stability in it, and it provided well-resolved absorption spectra without

interference. During wavelength selection, the standard solutions of both drugs were scanned in the range of 200–400 nm using methanol as the blank. The absorption maxima (λmax) were observed at 378 nm for Lornoxicam and 368 nm for Thiocolchicoside. The obtained spectra showed minimal overlap at these wavelengths, confirming that both drugs could be accurately quantified without mutual interference. The method exhibited excellent linearity for both drugs within the concentration range of 4–20 µg/mL and 5-25µg/mL respectively. The regression analysis yielded correlation coefficients (R^2) of 0.999, confirming strong linear relationships between absorbance and concentration. The precision of the method, determined through intra-day and inter-day studies, showed %RSD values less than 2%, indicating high repeatability and reproducibility of the results. Accuracy studies conducted by the standard addition method at 50%, 100%, and 150% levels demonstrated recoveries within 99–101%, validating the accuracy of the method and confirming that formulation excipients did not interfere with the estimation. The LOD and LOQ were found to be 0.09 µg/mL and 0.27 µg/mL for Lornoxicam, and 0.12 µg/mL and 0.36 µg/mL for Thiocolchicoside, (9) signifying the high sensitivity of the proposed method. Additionally, the robustness and ruggedness results indicated that minor variations in analytical conditions, such as wavelength and solvent composition, did not significantly affect the absorbance readings, with %RSD values remaining below 2%. All validation parameters met the acceptance criteria as per ICH Q2(R1) guidelines, confirming that the developed method is reliable, sensitive, and suitable for routine quality control analysis of pharmaceutical formulations containing both Lornoxicam and Thiocolchicoside.

CONCLUSION

The developed UV spectrophotometric method for simultaneous estimation of Lornoxicam and Thiocolchicoside is simple, precise, and accurate. The method showed excellent linearity and sensitivity, with low LOD and LOQ values confirming its suitability for trace analysis. Accuracy, precision, robustness, and ruggedness results meet ICH Q2(R1) acceptance criteria. Hence, this method can be successfully applied for routine quality control analysis of pharmaceutical formulations containing these two drugs.

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